981242

# PATENT SPECIFICATION

NO DRAWINGS

981,242

Date of filing Complete Specification Oct. 4, 1962.

Application Date Oct. 19, 1961. No. 37620/61.

Complete Specification Published Jan. 20, 1965.



Index at acceptance: —A5 B8

Int. Cl.:—A 61 k

## COMPLETE SPECIFICATION

## Vaccines Containing Rare Earth Salts

We, THE WELLCOME FOUNDATION LIMITED, of 183—193, Euston Road, London, N.W.1, a company incorporated in England, do hereby declare the invention, a communication from Cooper, McDougall & Robertson Limited, a company incorporated in New Zealand, of 731—733, Great South Road, Otahuhu, New Zealand, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

The present invention relates to vaccines and to the manufacture thereof.

In medicine, vaccines are useful for the prevention and treatment of diseases due to pathogenic microorganisms such as bacteria and viruses. They are especially useful in the technique known as active immunisation, whereby antibody formation is stimulated in an animal or a human by the administration of a vaccine containing antigenic material of microbial origin, which while conferring immunity does not cause the disease. Such a vaccine frequently contains antigenic material derived from the pathogenic microorganism and made nontoxic by toxoiding, for example with formaldehyde. A vaccine may contain antigenic material immunising against more than one type of pathogen, and is then known as a multivalent vaccine.

To increase and prolong the immunity given by a univalent or multivalent vaccine, there is often included in the vaccine an adjuvant to enhance its antigenicity. This sometimes takes the form of a precipitate containing an inorganic cation, which removes the antigenic material from solution by adsorption as an adjuvant-antigen complex. The salts of aluminium and in particular potash alum have been used as adjuvants in a great variety of vaccines.

[*Price* 4*s*. 6*d*.]

It has now been found that the rare earth cations enhance the antigenicity of vaccines and are useful as adjuvants. Of these cations lanthanum and cerium are readily available and especially effective.

Vaccines containing rare earth adjuvants are at least as good immunologically as those containing potash alum, and have several advantages. For example, tissue damage at the site of injection of a vaccine containing a rare earth adjuvant is less than that obtained with the same vaccine containing the equivalent quantity of potash alum as adjuvant. Furthermore, the concentration of the rare earth salt required to enhance the antigenicity of the vaccine is less than the concentration of potash alum required to give the same effect. Vaccines containing rare earth adjuvants generally remain in suspension for longer periods than the same vaccines containing potash alum as adjuvant, so facilitating their use as well as giving more elegant products.

The anion associated with the rare earth cation may be any immunologically acceptable anion; a convenient anion is acetate.

Rare earth adjuvants are particularly useful for enhancing the antigenicity of vaccines containing antigenic material of anaerobic bacterial origin. For example, they may be used in univalent or multivalent vaccines for the prevention of diseases due to *Clostridium botulinum, Clostridium chauvoei, Clostridium oedematiens, Clostridium perfringens* Types B, C and D, *Clostridium septicum* and *Clostridium tetani*. All these are important pathogens in veterinary medicine and some are also important in human medicine.

Rare earths adjuvants may also be used to enhance the antigenicity of vaccines for the prevention of diseases due to aerobic bacteria and viruses, for example *Corynebacterium*

*diphtheriae*, *Erysipelothrix rhusiopathia* and *Pasteurella septica*.

A vaccine containing a rare earth adjuvant may be parenterally administered in any manner known for the administration of vaccines; for example it may be injected subcutaneously, intradermally or intramuscularly.

The quantity of the rare earth cation required to enhance the antigenicity of a vaccine will depend on a number of variable factors, such as the nature of the vaccine, the manner of its preparation and the number of types and the amount of antigenic material in the vaccine. Thus 100 ml. of vaccine may contain between about 2 g. and about 0.008 g. of rare earth cation. The preferred quantity is about 0.2 g. of rare earth cation per 100 ml. of vaccine, which is given by about 0.5 g. of lanthanum acetate or cerium acetate per 100 ml. of vaccine.

A vaccine containing a rare earth adjuvant may be prepared by adding the rare earth cation to the vaccine as an aqueous solution of one of its salts, or the vaccine may be added to a water-soluble salt of the cation in the solid state. Alternatively, the vaccine may be added to a preformed precipitate containing the cation as a water-insoluble salt. The pH of the vaccine is finally adjusted to the optimum value for its antigenic components.

The present invention therefore provides a method of enhancing the antigenicity of vaccines containing antigenic material of microbial origin which comprises mixing with the vaccine a salt containing a rare earth cation associated with an immunologically acceptable anion.

The following examples illustrate the invention.

EXAMPLE 1—PULPY KIDNEY VACCINE

A 10% w/v aqueous solution of lanthanum acetate ($La(C_2H_3O_2)_3 . 1\frac{1}{2}H_2O$) was added to a solution of epsilon toxoid of pH 6.0—6.5, derived from a strain of *Clostridium perfringens* Type D, to give a final concentration of 0.5 g. of the salt per 100 ml. of vaccine. A precipitate was formed which contained most of the toxoid. The pH was adjusted to 5.8—6.2. The vaccine was preserved by 0.1% w/v thiomersal (sodium *o*-(ethylmercurithio)-benzoate).

EXAMPLE 2—BRAXY VACCINE

A 10% w/v aqueous solution of lanthanum acetate was added to a toxoided preparation of pH 7.0 derived from *Clostridium septicum*, giving a final concentration of 0.5 g. of the salt per 100 ml. of vaccine. The pH fell to 6.3 and a precipitate was formed which contained most of the antigenic material. The pH was adjusted to 5.8—6.2. The vaccine was preserved by 0.01% w/v thiomersal.

EXAMPLE 3—BLACKLEG VACCINE

A formaldehyde-treated culture of *Clostridium chauvoei* was mixed with a 10% w/v aqueous solution of lanthanum acetate to give a vaccine containing 0.5 g. of the salt in 100 ml. of vaccine. A precipitate was formed which contained most of the antigenic material. The pH was adjusted to 5.8—6.2. The vaccine was preserved by 0.01% w/v thiomersal.

EXAMPLE 4—PULPY KIDNEY VACCINE

A solution of 7.8 g. of lanthanum acetate in 250 ml. of water was mixed with a solution of 4.8 g. of aluminium chloride in 250 ml. of water. A solution of 8 ml. of concentrated ammonia in 250 ml. of water was added. The precipitate of mixed lanthanum and aluminium hydroxides was spun down immediately and washed with water. It was suspended in 250 ml. of water and dialysed (final volume 320 ml.) and autoclaved. Eight parts by volume of this autoclaved suspension were mixed with ten parts of a solution of epsilon toxoid derived from *Clostridium perfringens* Type D. The vaccine was preserved by 0.01% w/v thiomersal.

EXAMPLE 5—PULPY KIDNEY VACCINE

A 10% w/v aqueous solution of cerous acetate was added to a solution of epsilon toxoid of pH 6.0—6.5, derived from a strain of *Clostridium perfringens* Type D. A series of vaccine-adjuvant mixtures was prepared in which the final concentrations ranged from 0.2 g. to 2.0 g. of the salt per 100 ml. of vaccine. About optimal precipitation, measured by the degree of adsorption of epsilon toxoid, occurred when the final concentration was 0.5 g. of the salt per 100 ml. of vaccine. The pH was adjusted to 5.8—6.2. The vaccine was preserved by 0.01% w/v thiomersal.

EXAMPLE 6—PULPY KIDNEY VACCINE

A 10% w/v aqueous solution of cerous nitrate ($Ce(NO_3)_3 . 6H_2O$) was added to a solution of epsilon toxoid of pH 6.0—6.5, derived from a strain of *Clostridium perfringens* Type D, to give a final concentration of 0.5 g. of the salt per 100 ml. of vaccine. The pH was adjusted to 5.8—6.2. The vaccine was preserved by 0.01 w/v thiomersal.

EXAMPLE 7—BRAXY/BLACKLEG/PULPY KIDNEY VACCINE

A 10% w/v aqueous solution of lanthanum acetate was mixed with a trivalent preparation of antigenic material derived from *Clostridium septicum*, *Clostridium chauvoei* and *Clostridium perfringens* Type D, to give

a final concentration of 0.5 g. of the salt per 100 ml. of vaccine. The pH was adjusted to 5.8—6.2. The vaccine was preserved by 0.01% thiomersal.

EXAMPLE 8—BRAXY/BLACKLEG/PULPY KIDNEY VACCINE

A 10% w/v aqueous solution of cerous acetate was mixed with a trivalent preparation of antigenic material derived from *Clostridium septicum*, *Clostridium chauvoei* and *Clostridium perfringens* Type D, to give a final concentration of 0.5 g. of the salt per 100 ml. of vaccine. The pH was adjusted to 5.8—6.2. The vaccine was preserved by 0.01% thiomersal.

EXAMPLE 9—COMBINED VACCINE FOR DISEASES OF SHEEP

A seven-valent preparation of antigenic material derived from *Clostridium perfringens* Types B, C and D, *Clostridium septicum*, *Clostridium chauvoei*, *Clostridium oedematiens* and *Clostridium tetani* was added to autoclaved solid lanthanum acetate to give a final concentration of 0.5 g. of the salt per 100 ml. of vaccine. The pH was adjusted to 5.8—6.2. The vaccine was preserved by 0.01% thiomersal.

*Note* Although cerium cation was added as cerium (III), the final vaccine probably contained much cerium (IV).

WHAT WE CLAIM IS:—

1. A method of enhancing the antigenicity of vaccines containing antigenic material of microbial origin, which comprises mixing with the vaccine salt containing a rare earth cation associated with an immunologically acceptable anion.

2. A method claimed in Claim 1 wherein the antigenic material is of an aerobic bacterial origin.

3. A method claimed in Claim 1 or Claim 2, wherein the salt containing a rare earth cation is included to a concentration between about 2 g. and about 0.008 g. of the rare earth cation per 100 ml. of vaccine.

4. A method claimed in Claim 3, wherein the salt of the rare earth cation is included to a concentration of about 0.2 g. of cation per 100 ml. of vaccine.

5. A method claimed in any of Claims 1 to 4, wherein the rare earth salt is a lanthanum compound.

6. A method claimed in any of Claims 1 to 4, wherein the rare earth salt is a cerium compound.

7. A method claimed in either of Claims 5 and 6, wherein the rare earth salt is the acetate of lanthanum or cerium.

8. A vaccine whose antigenicity has been enhanced by a method as claimed in any of Claims 1 to 7.

9. A vaccine which contains antigenic material of microbial origin and an adjuvant comprising a salt containing a rare earth cation associated with an immunologically acceptable anion.

10. A vaccine claimed in Claim 9 which contains between about 2 g. and about 0.008 g. of the rare earth cation per 100 ml. of vaccine.

11. A vaccine claimed in Claim 10 which contains about 0.2 g. of the rare earth cation per 100 ml. of vaccine.

12. A vaccine claimed in any of Claims 9 to 11 in which the antigenic material is of anaerobic bacterial origin.

13. A vaccine claimed in any of Claims 9 to 12 in which the rare earth cation is lanthanum.

14. A vaccine claimed in any of Claims 9 to 12 in which the rare earth cation is cerium.

15. A vaccine containing a rare earth cation associated with an immunologically acceptable anion, substantially as herein described with reference to the examples.

16. A method for the prevention of diseases of animals other than man due to pathogenic microbes, which comprises stimulating antibody formation by the parenteral administration to the animal of a vaccine containing antigenic material of microbial origin and an adjuvant comprising a salt of a rare earth cation associated with an immunologically acceptable anion.

17. A method claimed in Claim 16 wherein the vaccine administered contains an adjuvant comprising a salt of the lanthanum cation associated with an immunologically acceptable anion.

18. A method claimed in Claim 16 wherein the vaccine administered contains an adjuvant comprising a salt of the cerium cation associated with an immunologically acceptable anion.

R. F. HASLAM,
Agent for the Applicants,
Chartered Patent Agent.

Leamington Spa: Printed for Her Majesty's Stationery Office by the Courier Press.—1965.
Published at The Patent Office, 25, Southampton Buildings, London, W.C.2, from which copies may be obtained.